

Pharmacokinetics of primaquine in man.

I. Studies of the absolute bioavailability and effects of dose size

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1 The pharmacokinetics of primaquine have been examined in five healthy volunteers who received single oral doses of 15, 30 and 45 mg of the drug, on separate occasions. Each subject received an i.v. tracer dose of [¹⁴C]-primaquine (7.5 µCi), simultaneously with the 45 mg oral dose.

2 Absorption of primaquine was virtually complete with a mean absolute bioavailability of 0.96 ± 0.08 .

3 Elimination half-life, oral clearance and apparent volume of distribution for both primaquine and the carboxylic acid metabolite were unaffected by either dose size, or route of administration.

4 The relationships between area under the curve and dose size were linear for both primaquine ($r = 0.99$, $P \leq 0.01$) and its carboxylic acid metabolite ($r = 0.99$, $P \leq 0.01$).

5 The mean whole blood to plasma concentration ratios were determined for primaquine (0.81), and for the carboxylic acid metabolite of primaquine (0.84).

6 Primaquine is a low clearance compound ($CL = 24.2 \pm 7.4 \text{ l h}^{-1}$), is extensively distributed into body tissues ($V = 242.9 \pm 69.5 \text{ l}$) and is not subject to extensive first pass metabolism.

Keywords primaquine pharmacokinetics bioavailability

Introduction

The antimalarial 8-aminoquinoline derivative, primaquine, has been in clinical use for over 40 years; however, little is known of its pharmacokinetics, particularly in relation to the influence of dose size. The importance of such pharmacokinetic information is underlined by the fact that, therapeutically, primaquine is used in oral daily doses ranging from 15 to 45 mg.

Although the pharmacokinetics of primaquine, after 45 mg oral dosage, has been examined recently (Greaves *et al.*, 1980; Mihaly *et al.*, 1984) neither its disposition after smaller single doses nor its absolute bioavailability have been studied. This is, in part, the consequence of

assay limitations which have now been overcome by the recent development of a simple, sensitive (1 ng/ml) selective and reproducible h.p.l.c. method of analysis (Ward *et al.*, 1984). In addition, a study of the absolute bioavailability of primaquine, requires its administration by the intravenous route. It has been proposed that primaquine is subject to extensive first pass hepatic extraction (Greaves *et al.*, 1980). This implies that i.v. dosage of this drug would be associated with substantially higher plasma drug concentrations than those seen with oral dosage and this could lead to greater pharmacological, and possibly toxic, effects after i.v. administration.

Therefore, the purpose of this study was firstly to examine the pharmacokinetics of primaquine over the therapeutic dosage range (15–45 mg) and secondly, to measure its absolute bioavailability. The latter was done by the simultaneous administration of [^{14}C]-primaquine i.v. (7.5 μCi ; 1.55 mCi/mmol) and a standard 45 mg oral dose, thus eliminating the possibility of intra-subject day to day variation in disposition.

Methods

Chemicals

Primaquine diphosphate was supplied by Aldrich Chemicals Ltd, Gillingham, Dorset, UK, 8-(3-amino-1-methylpropylamino)-6-methoxyquinoline was a gift from the Walter Reed Army Medical Research Centre, Washington D.C., USA and the carboxylic acid metabolite of primaquine was a gift from Professor J. McChesney, Department of Pharmacognosy, School of Pharmacy, University of Mississippi, USA. Indomethacin was obtained from Merck Sharp and Dohme Research Laboratories, Hertfordshire, UK and [^{14}C]-primaquine (sp. act. 1.55 mCi/mmol) was synthesized by New England Nuclear, Boston Mass., USA. Primaquine tablets (each containing 7.5 mg primaquine base) were obtained from ICI Pharmaceuticals, Alderley Edge, UK whereas [^{14}C]-primaquine for injection was prepared freshly in sterile normal saline (4.5 mg/5 ml; 1.5 $\mu\text{Ci}/\text{ml}$) which was passed through an in line, disposable, millipore filter (0.2 μm) during drug administration. Solvents were of h.p.l.c. grade and were obtained from Fisons, Loughborough, UK. All other reagents were of analytical grade and were supplied by B.D.H. Chemicals Ltd, Poole, Dorset, UK.

Volunteer study

Five healthy male volunteers (24 to 46 years) who were taking no other drugs, participated in this study. On separate occasions (at least 1 week apart) in randomized order and after an overnight fast each subject received oral primaquine 15, 30 and 45 mg (as the base). The 45 mg oral dose was accompanied by an intravenous dose of [^{14}C]-primaquine (7.5 μCi ; equivalent to 4.5 mg primaquine base in 5 ml solution), administered over 8 min. Venous blood samples (10 ml), for plasma drug assays, were collected pre-dose and again at 0.5, 1, 2, 3, 4, 6, 8, 10, 14 and 24 h post-dose and also at 10 and 17 min after the start of the intravenous dose. Blood was

centrifuged (1000 g for 15 min) and the separated plasma stored at -20°C until analysis. In the simultaneous i.v./oral dose study, additional samples (5 ml) for whole blood drug assays were taken at 2 and 6 h post dose and stored as for plasma.

All aspects of these protocols were approved by the Mersey Regional Health Authority Ethics Committee and the Ethics Committee of the World Health Organisation. Permission for the administration of [^{14}C]-primaquine was obtained from the D.H.S.S. Radioisotopes Panel (licence no. RPC283-1[4]).

Chromatography

All chromatographic analysis was carried out on a Spectra Physics Liquid Chromatograph. The system consisted of an SP8700 Solvent Delivery system, with an SP8750 organiser module, equipped with a Rheodyne valve injection system. Chromatographic separation was carried out on a $\mu\text{Bondapak}$ 'Rad-Pak' phenyl reversed phase column housed in a 'Z-module' and equipped with a 'CN-Guard Pak' pre-column (Waters Associates, Hartford, Cheshire, UK). Detection was by u.v. absorption at 254 nm.

Assay methods

(a) Primaquine and the carboxylic acid metabolite of primaquine: Plasma and whole blood concentrations of primaquine and the carboxylic acid metabolite of primaquine, were determined by selective and sensitive h.p.l.c. methods (Ward *et al.*, 1984; Mihaly *et al.*, 1984).

(b) [^{14}C]-Primaquine and the [^{14}C]-carboxylic acid metabolite of primaquine: Plasma, and whole blood levels of [^{14}C]-primaquine and [^{14}C]-carboxylic acid metabolite of primaquine, derived from the simultaneous i.v./oral dose study were determined as follows. After h.p.l.c. analysis, the eluted fractions corresponding to primaquine and the carboxylic acid metabolite of primaquine were collected and the radioactive content determined by liquid scintillation counting. The absolute concentrations of [^{14}C] radioactivity in each sample were adjusted in order to account for differences in extraction efficiency between samples. Extraction efficiency in a sample of concentration C, was calculated as follows:

$$\text{Extraction efficiency (\%)} = \frac{C(\text{ng/ml}) \times Y(\text{mm/ng})}{P(\text{mm/ml})} \times 100$$

Where Y is the magnitude of the recorder deflection equal to 1 ng of compound and P is the peak height of compound obtained per ml, from that

extracted sample. The absolute [^{14}C] concentration associated with each compound, per ml of sample, was then calculated as follows:

$$\frac{\text{Absolute } [^{14}\text{C}] \text{ concentration/ml}}{\frac{\text{Observed } [^{14}\text{C}] \text{ radioactivity/ml}}{\text{Extraction efficiency}}} \times \frac{100}{1}$$

(c) [^{14}C]-radioactivity:

Plasma levels of [^{14}C] radioactivity were determined by liquid scintillation counting using an Intertechnique SL33 liquid scintillation counter.

Pharmacokinetic calculations

The concentrations of primaquine and its carboxylic acid metabolite, reported here, for the 45 mg oral dose study, represent only non-radioactive drug concentrations, as the contribution to these concentrations of drug and metabolite, respectively, from the i.v. [^{14}C] dose, was subtracted at each time point.

The peak plasma concentration (C_{\max}) and the time at which it was reached (t_{\max}), the terminal phase elimination rate constant (λ_z), the terminal phase half-life ($t_{1/2}$), the area under the plasma concentration-time curve from time = 0 to 24 h AUC(0,24), the area under the curve from time 0 to infinity (AUC), oral clearance (CL_o), systemic clearance (CL), and the volume of distribution (V) were all calculated, as previously described (Mihaly *et al.*, 1984), by standard model independent pharmacokinetic methods (Gibaldi & Perrier, 1975; Rowland & Tozer, 1980).

The bioavailability (F) of primaquine was determined using the data from the simultaneous i.v./oral study substituted in the following expression

$$F = \frac{\text{AUC}_{\text{p.o.}}}{\text{AUC}_{\text{iv}}} \times \frac{\text{Dose}_{\text{iv}}}{\text{Dose}_{\text{p.o.}}}$$

This calculation assumes that clearance of primaquine is the same for the different sized i.v. and oral doses.

The whole blood to plasma distribution ratio (B/P) was calculated from the ratio of concentrations of compound in blood to those in plasma.

Statistical calculations

Statistical comparisons between two groups were made by Student's paired *t*-test. When multiple comparisons between more than two groups (of paired data) were made, two factor analysis of variance was used.

Data are tabulated as mean \pm s.d. and graphically as mean \pm s.e. mean. Statistical significance was accepted when $P \leq 0.05$.

Results

After a single oral 15 mg dose of primaquine, drug absorption was rapid, with peak plasma drug concentrations attained within 3 h (Figure 1). Thereafter plasma concentrations fell rapidly and monoexponentially with a mean elimination half-life of 5.9 ± 2.1 h. The mean AUC was $0.5 \pm 0.1 \mu\text{g ml}^{-1} \text{ h}$, the oral clearance was $31.2 \pm 7.01 \text{ h}^{-1}$ and the apparent volume of distribution was $269.2 \pm 120.9 \text{ l}$. The administration of 30 and 45 mg oral doses of primaquine was associated with proportional increases in AUC ($1.2 \pm 0.2 \mu\text{g ml}^{-1} \text{ h}$ and $1.7 \pm 0.4 \mu\text{g ml}^{-1} \text{ h}$, respectively). This linear rise in AUC ($r = 0.99$; $P \leq 0.01$) was not accompanied by any significant alteration in the values for the elimination half life, clearance or volume of distribution for primaquine (Table 1).

The carboxylic acid metabolite of primaquine, previously identified as the major plasma metabolite in man (Mihaly *et al.*, 1984) was detected in plasma within 30 min of dosing. By 4 h in each

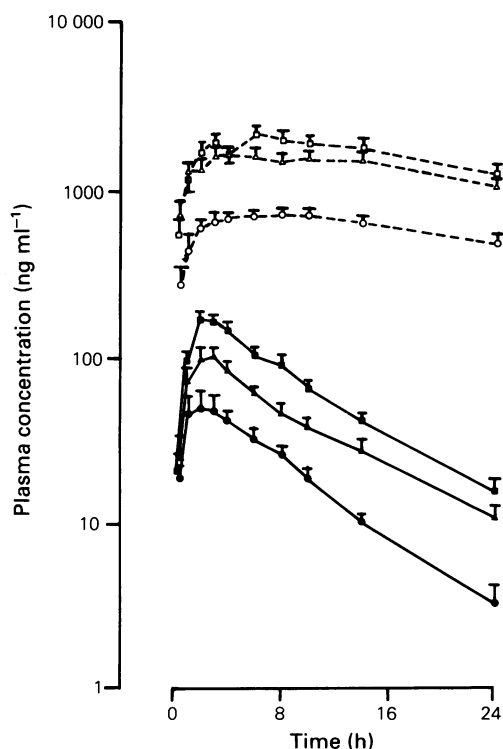


Figure 1 Semilogarithmic plot of plasma primaquine concentrations against time after oral primaquine; (15 mg (●—●), 30 mg (▲—▲) and 45 mg (■—■) and of corresponding plasma concentrations of the carboxylic acid metabolite after the 15 mg (○---○) 30 mg (△---△) and 45 mg (□---□) doses.

Table 1 Summary of pharmacokinetic parameters for primaquine after oral primaquine (15 and 30 mg) and simultaneous intravenous [14 C]-primaquine (7.5 μ Ci; 1.55 mCi/mmol; 4.5 mg) to five healthy subjects. The parameters are: maximum plasma concentration (C_{max}), time to reach maximum levels (t_{max}), oral clearance (CL_o), systemic clearance (CL), elimination half-life ($t_{1/2}$), volume of distribution (V_d), area under the curve from time = 0 to 24 h ($AUC(0,24)$) and area under the curve from time = 0 to infinity (AUC). Also shown are the values for $AUC(0,24)$ for the carboxylic acid metabolite of primaquine ($PQCO_2H$).

Dose (mg)	Route	C_{max} (ng ml $^{-1}$)	t_{max} (h)	CL_o (l h $^{-1}$)	CL (l/h)	$t_{1/2}$ (h)	V (l)	$AUC(0,24)$ (μ g ml $^{-1}$ h)	AUC (μ g ml $^{-1}$ h)	$AUC(0,24)$ ($PQCO_2H$) (μ g ml $^{-1}$ h)
15	oral	53 \pm 25	2 \pm 1	31.2 \pm 7.0	—	5.9 \pm 2.1	269 \pm 120	0.5 \pm 0.1	0.5 \pm 0.1	15.1 \pm 2.6
30	oral	104 \pm 25	3 \pm 1	27.3 \pm 5.3	—	7.4 \pm 2.5	281 \pm 69	1.0 \pm 0.2	1.2 \pm 0.2	33.4 \pm 4.2
45	oral	176 \pm 43	2 \pm 1	27.1 \pm 5.5	—	6.7 \pm 0.5	263 \pm 57	1.6 \pm 0.4	1.7 \pm 0.4	39.9 \pm 6.1
4.5 (7.5 μ Ci)	i.v.	—	—	—	24.2 \pm 7.4	7.1 \pm 1.3	243 \pm 69	0.2 \pm 0.1	0.2 \pm 0.1	4.1 \pm 0.8

study, plasma concentrations of this compound were more than 10 fold greater than those attained by primaquine. Despite falling primaquine concentrations, the concentrations of the carboxylic acid metabolite were maintained at their elevated level, throughout the remainder of each study (Figure 1). The proportion of the primaquine dose converted to the carboxylic acid metabolite was unaffected by dose size, as illustrated by the linear increase in the $AUC(0,24)$ for this metabolite with increasing dose of parent drug ($r = 0.99$; $P \leq 0.01$; Table 1). Consequently, the ratio of $AUC(0,24)$ obtained for the carboxylic acid metabolite to $AUC(0,24)$ obtained for primaquine was similarly unaffected by dose size and was approximately 30:1 in each case (Table 1).

After simultaneous i.v. and oral administration of primaquine there was no significant difference between the values for the half-life, AUC , clearance and volume of distribution (Figure 2; Table 1). From these data the absorption of orally administered primaquine was shown to be virtually complete as the calculated value for bioavailability approached unity in every subject (Table 2).

Plasma total [14 C] concentrations fell sharply but only transiently after i.v. administration of [14 C]-primaquine (Figure 3). However, after 30 min plasma [14 C] radioactivity rose until at 6 h the levels of radioactivity plateaued at 0.04% of the dose/ml of plasma. Over the 24 h period of

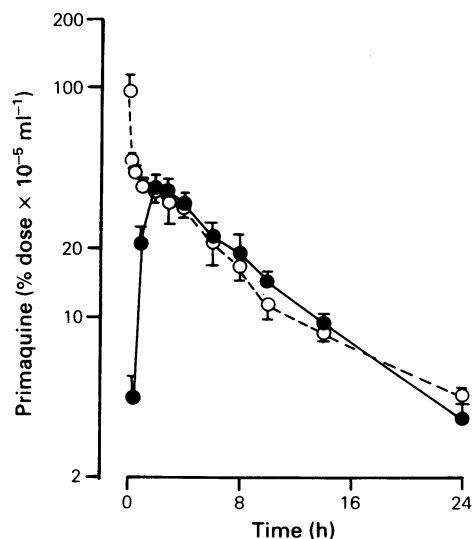


Figure 2 Semilogarithmic plot of plasma primaquine concentrations against time after simultaneous intravenous (○---○; 7.5 μ Ci, 1.55 mCi/mmol, 4.5 mg) and oral (●—●; 45 mg) dosage.

Table 2 Individual areas under the plasma concentration time curve (AUC) for primaquine after simultaneous i.v. and oral administration. Bioavailability (F) was determined from the ratio of AUCs after oral and i.v. dosage

Subject	AUC (% dose ml ⁻¹ h × 10 ⁻⁵)		F
	oral	i.v.	
AB	313.6	330.7	0.95
GM	561.0	589.1	0.95
GE	336.4	302.2	1.10
MS	370.6	417.4	0.89
SW	528.4	573.6	0.92
Mean	422.0	442.6	0.96
± s.d.	114.4	133.7	0.08

the study 38% of the plasma [¹⁴C]-radioactivity AUC was accounted for by plasma concentrations of the [¹⁴C]-carboxylic acid metabolite, whereas less than 5% was due to the parent drug.

The whole blood to plasma distribution (B/P) ratios of primaquine, and the carboxylic acid metabolite of primaquine were determined at 2 and 6 h after the simultaneous i.v./oral dose study. There were no significant differences in B/P in each individual, between the 2 and 6 h samples for primaquine (B/P at 2 h = 0.81 ± 0.11 and at 6 h = 0.80 ± 0.14), and for the carboxylic acid metabolite of primaquine (B/P at 2 h = 0.84 ± 0.37 and at 6 h = 0.83 ± 0.40).

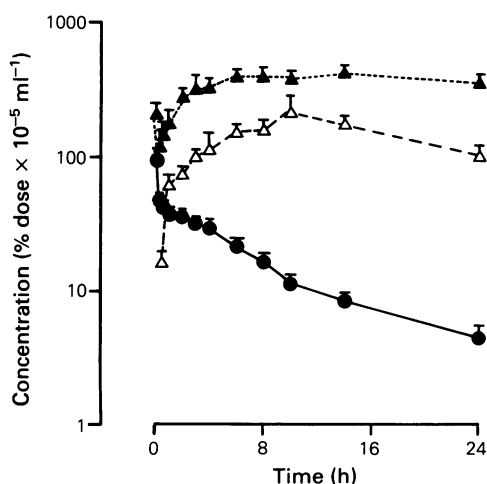


Figure 3 Semilogarithmic plot of total [¹⁴C]-radioactivity (▲---▲), [¹⁴C]-primaquine (●---●) and of [¹⁴C]-carboxylic acid metabolite levels (Δ---Δ) obtained after intravenous dosage with [¹⁴C]-primaquine (7.5 μCi, 1.55 mCi/mmol, 4.5 mg).

Discussion

The therapeutic regimens for primaquine in the radical cure of malarial infections utilize doses ranging from 15–45 mg p.o. daily (Rollo, 1980). Pharmacokinetic studies to date have only examined the disposition of a single 45 mg oral dose of primaquine (Greaves *et al.*, 1980; Mihaly *et al.*, 1984). In the earlier of these studies the finding that primaquine achieved only low plasma concentrations was attributed to the presence of a low bioavailability and/or the presence of extensive first pass metabolism of primaquine (Greaves *et al.*, 1980). In addition, recent experimental evidence from this laboratory has demonstrated perfusion limited hepatic disposition of primaquine at low doses in the isolated perfused rat liver (Ward *et al.*, 1984b). However, detailed studies in man of the absolute bioavailability of primaquine and of its pharmacokinetics across the clinically used dosage range have not been undertaken.

The results of the present study demonstrated that the pharmacokinetics of primaquine, after oral administration, are unaffected by dose size within the clinically used dosage range (Figure 1; Table 1). Therefore, as would be expected, the extent of formation of the carboxylic acid metabolite of primaquine (as measured by the ratios of AUCs for this metabolite and the parent drug) was also unaffected by dose size (Figure 1; Table 1). These results are in contrast to those cited in the primaquine disposition studies referred to above, and to those found using the isolated perfused rat liver (Ward *et al.*, 1985). In the latter, primaquine doses in the range of 0.5 to 5.0 mg were associated with marked differences in the perfusate pharmacokinetics, metabolic fate and extent of biliary excretion of the drug. This discordance in results between man and rat may, in part, be due to the much higher concentrations of primaquine in the perfusate compared with human plasma or to intrinsic differences between species in the disposition of the drug.

The extent of absorption of primaquine was shown to be virtually complete in the present study, as illustrated by the near unity values for the absolute bioavailability of this drug (Table 2). As the i.v. and oral doses had been administered simultaneously in this part of the study, the variance in the estimates for bioavailability was low. This advantage is achieved because intra subject day to day variation in drug disposition is avoided by concurrent administration of the i.v. and oral doses. In addition, the systemic clearance of primaquine was determined from the i.v. dose study (CL = 24.2 ± 7.4 l h⁻¹), confirming that this drug is a low to intermediate

clearance compound and that low systemic drug levels obtained after oral dosage are due to the rapid and extensive tissue distribution of the drug ($V = 242.9 \pm 59.5$ l) and not due to avoid hepatic elimination as previously suggested (Greaves *et al.*, 1980).

Following the intravenous administration of [^{14}C]-primaquine plasma concentrations of both [^{14}C]-primaquine and total [^{14}C] radioactivity declined sharply (Figures 2 and 3). However, although [^{14}C]-primaquine concentrations continued to fall, the levels of total radioactivity from 30 min post dose, climbed steadily before plateauing at 6 h at a level which was maintained for the remainder of the 24 h sampling period. Although this implies an indefinite persistence of [^{14}C] primaquine, we have previously noted that radioactivity declines from 24 h onwards and returns to background levels after several days (Mihaly *et al.*, 1984).

Approximately 40% of the total [^{14}C]-radioactivity AUC(0,24) in plasma was accounted for by the AUC(0,24) for the [^{14}C]-carboxylic acid metabolite. Furthermore the ratio of AUC(0,24) for this metabolite to the AUC(0,24) for primaquine was 25:1, which is similar to the ratios obtained after oral administration. These results confirm that the carboxylic acid metabolite is a principal plasma metabolite of primaquine, as has previously been reported (Mihaly *et al.*, 1984), and that the extent of formation of this metabolite is unaffected by the route of administration. Furthermore, as the AUC for the carboxylic acid derivative is greater than that of the parent drug, the clearance of the metabolite

must have been less than that for primaquine.

Some antimalarial agents, in particular the 4-aminoquinoline derivative chloroquine, have been shown to concentrate selectively in white and red blood cells resulting in whole blood: plasma concentration ratios of 10:1 (Bergqvist *et al.*, 1983). We found no evidence for any accumulation of primaquine, or the carboxylic acid metabolite in blood cells. This is in keeping with the exoerythrocytic mode of action of this antimalarial agent (Bruce-Chwatt, 1979). A detailed evaluation of the distribution characteristics of primaquine into specific blood cell components was not undertaken.

The present study showed that the pharmacokinetics of primaquine and of its carboxylic acid metabolite are independent of both dose size and route of administration and that neither drug nor its carboxylic acid metabolite undergo accumulation into blood cells—unlike other antimalarials. We also showed that primaquine is completely absorbed after oral administration into the systemic circulation and that, in contrast to earlier suggestions, this drug is a low clearance compound which is not subject to extensive first pass hepatic extraction.

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